

Stress-relevant social behaviors of middle-class male cynomolgus monkeys (*Macaca fascicularis*)

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ABSTRACT

Stress from dominance ranks in human societies, or that of other social animals, especially nonhuman primates, can have negative influences on health. Individuals holding different social status may be burdened with various stress levels. The middle class experiences a special stress situation within the dominance hierarchy due to its position between the higher and lower classes. Behaviorally, questions about where middle-class stress comes from and how individuals adapt to middle-class stress remain poorly understood in nonhuman primates. In the present study, social interactions, including aggression, avoidance, grooming and mounting behaviors, between beta males, as well as among group members holding higher or lower social status, were analyzed in captive male-only cynomolgus monkey groups. We found that aggressive tension from the higher hierarchy members was the main origin of stress for middle-class individuals. However, behaviors such as attacking lower hierarchy members immediately after being the recipient of aggression, as well as increased avoidance, grooming and mounting toward both higher and lower hierarchy members helped alleviate middle-class stress and were particular adaptations to middle-class social status.

Keywords: Stress; Social behaviors; Beta individual; Male-only; Cynomolgus monkey

INTRODUCTION

People with different socioeconomic status are thought to experience different levels of psychosocial stress, which can lead to various health problems such as physiological and metabolic alternations, disabilities, stress-related diseases and even mortality (Adler et al, 1993, 1994; Brunner, 1997; Manuck et al, 1995; Sapolsky, 2005; Shively et al, 2005). In addition to human studies, Abbott (2003) and colleagues conducted a

meta-review on dozens of nonhuman primate species, which showed strong correlations between social rank and stress level, and although the associations varied among species, certain ranks exhibited a corresponding amount of stress. Sapolsky (2005) also reviewed and reported on the close relationship between social status and health conditions in nonhuman primates, and concluded that social status markedly influenced health. Accordingly, investigations on social status and stress levels have drawn increasing attention.

Bupa claims that more than a half of middle-class managers suffer from overwhelming stress problems and experience mental health conditions such as depression (Bupa Research, 2013). Similarly, Edwards et al (2013) found that middle-class barbary macaques experience more social stress than those with relatively higher or lower social status. Thereby, middle-class stress seems to draw increasing attentions from general studies of graded variations between social rank and stress conditions. However, the origins of middle-class stress and how middle-ranked individuals adapt to their particular social status remain poorly understood.¹

In the present study, we selected beta monkeys in captive, male-only, cynomolgus monkey groups (as middle-ranked individuals), and observed and analyzed four types of social behaviors (aggression, avoidance, grooming and mounting) in the hope of clarifying the origins of middle-class stress and how beta monkeys adapt to their social status from a behavioral point of view.

MATERIAL AND METHODS

Subjects

Data were collected from seven isosexual social colonies of male cynomolgus monkeys (*Macaca fascicularis*), with 10-15

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individuals aged 4-5 years in each group. The monkeys were housed in colonies (3 m×5 m×3 m) at Hainan Jingang Biotech Co., Ltd. (Tian & Ma, 2014), and had free access to water and monkey chow supplemented with fruit and vegetables. The animals were reproductively intact. The members in each group were unchanged for at least two years prior to initial sampling. The treatment of the animals was conducted in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and was approved by the Biological Research Ethics Committee of the Hainan Jingang Biotech Co., Ltd.

Behavioral sampling

The monkeys were familiarized with observers until they were completely adapted to observers remaining in front of the cage. Observers stood or sat at a distance of at least 2.5 m from the cage. Except for taking sampling notes, observers did not move about freely or feed the animals. Actual behavior sampling started after the adaptation period.

Social status was determined via instant scan sampling (Altmann, 1974) during the adaptation period, and was based on the order of eating and the ability to defeat others in each group. More specifically and theoretically, the animal that defeated all other members in its group and ate first when food was provided was ranked as the alpha (top) male; the animal that defeated all but the alpha male was ranked as the beta (second) male; and so forth. In the present study, the top two ranked monkeys in each group were very clear, and their respective statuses were stable during the observation period. However, the ranks of the remaining individuals (excluding the lowest) were unclear and unstable because their observable behavioral differences were difficult to determine. Similar to human middle-ranked managers (Bupa Research, 2013), beta males had to obey the alpha males (boss) as well as maintain their beta status over the other group members. We therefore selected the middle-ranked beta males as the target individuals in this study as they were hierarchically positioned between the alpha and lower-ranked members.

The frequencies of social behaviors, including aggression, avoidance, grooming and mounting, were recorded using focal animal sampling (Altmann, 1974). The frequency of social behaviors between the beta male and other cage mates was recorded. From the action direction point of view, we recorded six sub-categories for each social behavioral type, namely, actions initiated by the alpha male toward the beta male, beta male toward the alpha male, alpha male toward 'others', 'others' toward the alpha male, beta male toward 'others', and 'others' toward the beta male. Each monkey group was recorded for three consecutive days in total, six times a day, 10 min per time between 1700h and 1900h.

Statistical analysis

Student's *t*-tests (paired) were used for comparison of the frequency (over entire observation period) differences among sub-categories in each social behavioral category. The *t*-tests were two-tailed with *P*<0.05 considered to be statistically significant.

RESULTS

Aggressive behavior

No aggressive behavior, across all seven monkey groups and the entire observation period, was initiated by the beta male or 'others' toward the alpha male, nor from 'others' toward the beta male. Aggression mainly occurred between the alpha male and beta male/'others', as well as the beta male and 'others'. As shown in Table 1, the average frequency of the alpha male attacking the beta male or 'others' was 3.14 and 2.57, respectively. The beta males received more than half (55%, 22 out of 40 occurrences) the aggressive actions initiated by the alpha males.

In addition, compared with the alpha male, the beta male attacked the 'others' more frequently (average frequency of 2.57 v.s. 7.00, *P*=0.0008, *t*=6.16, *SD*=1.9). The beta male was attacked by the alpha male on 72.7% of occasions (16 out of 22 times per group), after which the beta male immediately initiated aggressive behavior toward the 'others'. By comparing the aggressive behaviors received (A to B, 3.14 times per group on average) and given by the beta male (B to 'others', 7.00 times per group on average), the beta male was more often an aggressor than victim (*P*=0.006, *t*=4.12, *SD*=2.47).

Table 1 Frequencies of aggressive behavior

Group No.	A to B	A to others	B to others	A to all
1	3	3	9	6
2	2	0	3	2
3	3	2	4	5
4	3	1	5	4
5	3	5	8	8
6	4	5	11	9
7	4	2	9	6
Average	3.14	2.57	7	5.71
Sum	22	18	49	40
<i>P</i>		_____0.0008_____		
		_____0.006_____		

Animal's number from 1-7: the seven monkey groups, respectively; A and B: alpha male and beta male, respectively; *P*: values from student's *t*-tests, and the data pairs for comparison are the two columns indicated by the underscore at both sides of the number (i.e., the paired *t*-test of column "A to others" and "B to others" resulted in *P*=0.0008; similarly, the "A to B" and "B to others" columns showed significant differences with *P*=0.006); Abbreviations and *P* values are the same in Tables 2-4 below.

Avoidance behavior

Avoidance behavior occurred more frequently than the other behaviors, as seen from the total number of each sub-category (97, 68, and 89 times) shown in Table 2. The beta male avoided the alpha male more frequently than the 'others' did (13.86 v.s. 9.71 times per group on average, *P*=0.018, *t*=3.19, *SD*=3.43). There was no significant avoidance predilection of 'others' to either the alpha or beta males, although they showed more, in

Table 2 Frequencies of avoidance behavior

Group No.	B to A	Others to A	Others to B
1	13	12	14
2	10	9	12
3	12	7	11
4	12	7	13
5	23	20	11
6	17	6	19
7	10	7	9
Average	13.86	9.71	12.71
Sum	97	68	89
<i>P</i>	_____0.018_____	_____0.269_____	

total number (68 v.s. 89 times), avoidance behavior toward the beta males. The alpha males exhibited no avoidance behavior.

Grooming (affinitive behavior)

As shown in Table 3, grooming was dual directional among the alpha male, beta male and 'others'. The beta males groomed the alpha males much more frequently than the reverse (5.43 v.s. 1.00 times on average, $P=0.003$, $t=4.55$, $SD=2.57$). The beta males also groomed the alpha males much more frequently than they did the 'others' (5.43 v.s. 1.71 time on average, $P=0.003$, $t=4.6$, $SD=2.14$). We did not find any significant differences in the beta male receiving or providing grooming from/to the 'others'. This differed from that observed between the alpha males and 'others', namely, alpha males groomed the 'others' much less frequently than 'others' groomed the alpha males (0.71 v.s. 2.29 on average, $P=0.017$, $t=3.27$, $SD=1.27$).

Table 3 Frequencies of grooming behavior

Group No.	A to B	B to A	B to others	Others to B	A to Others	Others to A
1	2	3	1	2	0	2
2	2	6	0	4	0	3
3	1	4	1	1	1	3
4	1	4	0	3	0	1
5	0	5	2	1	2	2
6	0	9	8	0	0	0
7	1	7	0	3	2	5
Average	1	5.43	1.71	2	0.71	2.29
Sum	7	38	12	14	5	16
<i>P</i>	_____0.003_____	_____0.858_____	_____0.017_____			
		_____0.003_____				

Mounting behavior

Mounting was the least frequently performed behavior among the four categories of social behaviors. As shown in Table 4, the alpha males mounted the beta males a total of five times, with beta males only mounting an alpha male on one occasion in one group, but mounting the 'others' on seven occasions. No mounting behaviors were observed from the 'others' toward either the alpha or beta males. No significant differences were observed between any two sub-categories in regards to mounting behavior.

Table 4 Frequencies of mounting behavior

Group No.	A to B	B to A	B to others
1	2	0	2
2	1	0	1
3	0	0	1
4	1	1	1
5	0	0	0
6	1	0	1
7	0	0	1
Average	0.71	0.14	1
Sum	5	1	7

DISCUSSION

In the present study, we investigated two aspects relevant to the stress conditions of beta individuals in male-only cynomolgus monkey groups; namely, the origins of stress experienced by beta males and the adaptation of beta males to stress from their own social status. Observations of various social behavioral contacts between the beta male and his cage mates were conducted to clarify these questions from a behavioral point of view.

We suggested that the origin of stress for the beta male was mainly from being attacked by the alpha male. According to our results, the beta male received 55% of aggressive behavior initiated by the alpha male. As a single target, the beta male

received more aggression from the alpha male than the total number of attacks on the other 8-13 individuals. This strongly suggests that the beta male was the main target of aggression from the alpha male. Furthermore, a different research group in our lab found a significant positive correlation between aggressive behavior and hair cortisol (a stress hormone) concentration in adult male rhesus monkeys (unpublished data); namely, the more aggression an individual received, the higher their hair cortisol concentrations, and thus the higher their stress levels. Our results indicated that beta male stress mainly originated from aggressive pressure from the alpha male in each respective group.

Individuals with a certain social status have to adapt to their specific situations, including playing their expected role and dealing with stress. As stated previously, middle-ranked human managers have to obey their higher-ranked bosses, while also leading their lower-ranked subordinates, and thereby hold special status and high levels of stress. Similarly, in the present study, the beta male held a 'middle-class' rank, thus while obeying the alpha male, he also had to maintain his beta status in the group. How do beta males adapt to the stress of middle class? We found 72.7% of aggressions when the beta male was attacked by the alpha male, after which the beta male immediately initiated aggressive behavior toward the 'others'. The beta male also attacked 'others' more frequently than the alpha male did (7.00 v.s. 2.57 times per group on average, $P=0.0008$, $t=6.16$, $SD=1.9$). The intense aggressiveness of the beta male (similar rate as that of alpha males in total, 49 v.s. 40; and more frequently to 'others' than that of the alpha males, 49 v.s. 18, $P=0.0008$, $t=6.16$, $SD=1.9$) was likely performed to maintain his social status as the ability to defeat group members was a determinant of social rank; on the other hand, this aggressive behavior (as suggested from the 72.7% redirection of aggression by the beta male) may be a way to release high tension from being attacked by the alpha male. Previous studies have reported that social behaviors such as grooming, avoidance and mounting can serve as important functions to reduce the stress of opponents, as well as to help decrease the frequency of conflicts (i.e., Bernstein & Ehardt, 1985; Castles & Whiten, 1998; Cords, 1992; Das et al, 1998; Faraut et al, 2015). In particular, affiliative and reconciliation behaviors play a significant role in soothing stress and reducing conflict. Many studies suggest that to maintain a relatively balanced and peaceful social environment, after fighting or threatening one another, most animals show friendliness by touching, hugging or grooming one another (Aureli et al 1989; Cheney & Seyfarth, 1989; De Waal & Roosmalen, 1979; De Waal & Yoshihara, 1983; York & Rowell, 1988). Compared with the 'others', we found that beta males showed more avoidance behavior toward the alpha males (13.86 v.s. 9.71 times per group, on average, $P=0.018$, $t=3.19$, $SD=3.43$), and groomed the alpha male more frequently (5.43 v.s. 1.71 times, on average, $P=0.003$, $t=4.6$, $SD=2.14$). We considered these beta male avoidance and grooming behaviors as strategies to relieve stress from aggression and reduce potential conflicts with the alpha male. In addition, with no significant differences in frequencies (1.71 v.s. 2.00 times per group on average), the

grooming behaviors between beta and 'others' could also help with soothing stress of the beta male. Furthermore, although mounting behaviors between the beta and alpha males (six times in total)/'others' (seven times in total) were rare, they may have served to mitigate stress as well.

It is important to note that certain factors that may contribute to stress, such as breeding and survival issues, were controlled in our observation groups, which might also contribute to answering the questions of interest. Specifically, all monkey subjects had free access to food and water, which eliminated the stress for survival. They also had no visual contact with females, which reduced the effects of reproduction and breeding season. Therefore, beta male stress was most likely from social interactions with their male cage mates.

In conclusion, due to its middle rank within the hierarchical social group, the beta males in the captive male-only cynomolgus monkey groups experienced a special stressful situation due to aggressions from the higher-ranked alpha males. To reduce their stress and adapt to their beta status, the beta males were actively involved in social behaviors, i.e., aggression toward 'others' after being attacked, as well as receiving and performing avoidance, grooming and mounting behavior. Further physiological studies, such as comparison of plasma/hair cortisol levels among individuals holding different social statuses, should be conducted in combination with future behavioral analysis.

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